Journal of Environmental Quality TECHNICAL REPORTS
TRACE ELEMENTS IN THE ENVIRONMENT

The Mobility of Silver Nanoparticles and Silver Ions in the Soil-Plant System

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Abstract

The widespread use of silver nanoparticles (AgNPs) as a bactericide will ultimately result in their increased concentration in soils. We sought to determine the likely mobility, toxicity, and plant uptake of Ag applied to soil as either AgNPs or Ag^+. We measured the solubility, toxicity, and plant uptake of both AgNPs and Ag^+ in an immature Pallic soil, a Templeton loamy silt (pH = 5.1), and a granular silt loam (pH = 6.0). The sorption of AgNPs by the test soils was significantly greater than Ag^+, and both moieties were more strongly sorbed at lower concentrations and higher pH values. Between pH 4 and 8, distribution coefficient (K_D) values increased from <10 up to ~500 L kg^{-1} for Ag^+, and from 100 to 10,000 L kg^{-1} for AgNPs. There was strong evidence that our citrate-coated AgNPs were transformed into Ag^+ during the course of the plant growth experiments, and plant responses were similar for both the Ag^+ and AgNP treatments. Soil concentrations >100 mg kg^{-1} significantly reduced the biomass of *Lolium perenne* L. and resulted in foliar concentrations of up to 10 mg kg^{-1} dry matter. At a soil concentration of 70 mg kg^{-1}, silverbeet [*Beta vulgaris* L. ssp. *maritima* (L.) Arcang.] and spinach (*Spinacia oleracea* L.) accumulated 5 to 10 mg Ag kg^{-1}, which may present a human health risk. Regarding citrate-coated AgNPs, the environmental impact of release is largely determined by the equivalent mass concentration of Ag^+, into which they will ultimately transform. Given the widespread interest in AgNPs, there is limited knowledge on the behavior of Ag^+ in soil and this should be the subject of future research.

Core Ideas

- Citrate-coated Ag nanoparticles had greater soil sorption than Ag^+.
- Sorption of both Ag nanoparticles and Ag^+ increased at higher pH.
- Silver nanoparticles are ultimately unstable and transform to Ag^+.
- Phytotoxicity occurs at soil Ag concentrations >100 mg kg^{-1}.
- Silver uptake by spinach and silverbeet may present a human health risk.

J. Environ. Qual. 48:1835–1841 (2019)
doi:10.2134/jeq2019.03.0098
Supplemental material is available online for this article.
Received 3 Mar. 2019.
Accepted 1 July 2019.
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Silver nanoparticles (AgNPs) are increasingly used due to their antimicrobial activity. As of 2015, >410 products on the global market contained AgNPs, with an annual global production of >550 t (Hedberg et al., 2015). Household and industrial waste may enter soil via the land application of treated municipal effluent or sewage sludge (Colman et al., 2013) or via industrial emissions. Potentially, AgNPs may accumulate in soil, leading to reduced soil fertility (Shin et al., 2012; Peyrot et al., 2014), entry into food chains via plant uptake (Anjum et al., 2013), or leaching of Ag into groundwater.

Silver nanoparticles are insoluble, forming colloidal solutions when coated with citrate (Howe and Dobson, 2002). The behavior of AgNPs in soil depends on their size and surface coating (Dong et al., 2009). For example, citrate-coated AgNPs are more soluble and more persistent than their polyvinylpyrrolidone (PVP)-coated counterparts (McGillicuddy et al., 2017).

Through their surface coating, AgNPs may bind to soil colloids via specific adsorption (Pallavicini et al., 2014). As AgNPs may have a surface charge, they may also interact electrostatically with soil colloids, although Klitzke et al. (2015) reported that the surface charge had minimal effect on AgNPs sorption by soil. The degree of AgNP sorption in soil is affected by pH, ionic strength, and organic matter content, including the fraction of dissolved organic matter (Kleja et al., 2016; Van Koetsem et al., 2018). As with other reactive moieties, AgNP and Ag^+ sorption are higher in clay-rich soil than in sandy soil (Jacobson et al., 2005).

Biological or chemical removal of the surface coating of AgNPs allows the oxidation of the underlying zerovalent Ag in the nanoparticles. Ultimately, AgNPs transform into Ag^+ in the environment (Liu and Hurt, 2010). Silver ions (Ag^+) could enter soil solution from the oxidation of NPs or dissociation of adsorbed Ag from the soil (Benoit et al., 2013). Geochemically, Ag is classed as a chalcophile, often associated with sulfur (McLennan, 1998). The solubility and hence mobility of Ag^+ is limited by precipitation with chloride (Sagee et al., 2012), as well as the normal sorption processes affecting metal cations.

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Abbreviations: AgNP, silver nanoparticle; FAAS, flame atomic absorption spectrophotometer; GSL, granular silt loam; ICP–OES, inductively coupled plasma optical emission spectrometry; PVP, polyvinylpyrrolidone; TDI, tolerable daily intake; TLS, Templeton loamy silt.

In soil, AgNP concentrations as low as 0.14 mg Ag kg^{-1} can adversely affect soil biota (Colman et al., 2013). Cox et al. (2016) reported that AgNPs were more toxic than TiO_2, resulting in higher oxidative stress and genotoxicity, as well as lower seed germination and root elongation in *Arabidopsis thaliana* (L.) Heynh., *Oryza sativa* L., *Lemna gibba* L., *Ricinus communis* L., *Vigna radiate* (L.) R. Wilczek, *Cucurbita pepo* L. Exposure to >25 mg kg^{-1} of AgNPs and Ag^+ induces oxidative stress, similar to other trace elements (Kumari et al., 2009). The toxicity of AgNPs is size dependent, with smaller AgNPs (<20 nm in diameter) being more penetrative and toxic than larger particles (Scherer et al., 2019).

Silver nanoparticles are deposited on the surface of the cell and organelles, resulting in growth inhibition in some plants such as radish (*Raphanus sativus* L.) sprouts (Zuverza-Mena et al., 2016), and lettuce (*Lactuca sativa* L.) at solution concentrations of ~10 mg L^{-1} (Gruyer et al., 2014). Paradoxically, other studies have shown that low concentrations of AgNPs increase the growth of *Brassica* spp. and rocket (*Eruca sativa* Mill.) (Sharma et al., 2012; Yin et al., 2012; Vannini et al., 2013), possibly through hormesis (Mattson and Calabrese, 2010).

Most previous work on plant uptake has focused on hydroponic cultures because it enables the precise measurement of soluble Ag and AgNPs, which can be used as a proxy for bioavailable Ag. There is a dearth of information comparing AgNPs and Ag^+ on plant uptake from soil and relating this to Ag in soil solution, as estimated using a dilute-salt extraction. We hypothesized that as AgNPs and Ag^+ will be retained by soil, plant uptake will be limited, even at concentrations manifold higher than those used in the abovementioned hydroponic experiments.

We aimed to determine the solubility, toxicity, and plant uptake of both AgNPs and Ag^+ in two distinct soil types and elucidate the risk of AgNPs accumulating in soil, leaching to groundwater, or entering the food chain via plant uptake. We selected *Lolium perenne* L. for detailed investigation at several concentrations of Ag^+ and AgNPs, because this is one of the most commonly occurring pasture species (Cunningham et al., 1993). Nine edible plants (carrot [*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.], radish [*Raphanus raphanistrum* L. var. *sativus* (L.) G. Beck], leek [*Allium ampeloprasum* L.], lettuce, parsley [*Petroselinum crispum* (Mill.) Nyman ex A.W. Hill], rocket, beetroot [*Beta vulgaris* ssp. *vulgaris* L.], silverbeet [*Beta vulgaris* L. ssp. *maritima* (L.) Arcang.], and spinach [*Spinacia oleracea* L.]) were screened for Ag uptake when grown on soils spiked with AgNPs or Ag^+.

Materials and Methods

Soils

About 500 kg of a typic immature Pallic soil, a Templeton loamy silt (fine-silty, isotic, isomesic Andic Humudepts; TLS), was collected from a nongrazed section of the Lincoln University commercial Dairy Farm, New Zealand (43°38′11.35″ S, 172°26′17.00″ E). A granular silt loam (GSL) was collected from a commercial vegetable growing area in Pukekohe, New Zealand (37°13′18.92″ S, 174°52′5.94″ E). After removing nondecomposed plant material, soils were collected from the top 0.25 m. The soils were air dried and passed through a 7-mm sieve to remove large stones and roots. The soil was homogenized using a spade. A subsample (20 kg) of soil for the laboratory experiments was dried at 60°C until a constant weight was obtained and sieved to <2 mm using a nylon sieve. Table 1 gives the properties of the TLS and GSL.

Nanoparticle Preparation

Silver nanoparticles were freshly prepared on the day of the experiments. Citrate-coated AgNPs were synthesized from $AgNO_3$, reduced by $FeSO_4 \cdot 7H_2O$ (Sigma-Aldrich), and coated with $HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$ (Sigma-Aldrich) based on the method described by (Carey, 1889), with details in Supplemental Table S1. The zeta potential of AgNP was determined using a Zetasizer Nano ZS (Malvern Instruments) at 25°C, equal to −41 mV. The size of the AgNP was measured using a Philips CM200 high-resolution analytical transmission electron microscope fitted with a Gatan digital camera. Based on these measurements, the diameter distribution of the samples synthesized varied between 10 and 40 nm with an average diameter of 25 nm. The average-sized AgNP contains ~380,000 Ag atoms and has a mass of 41,000,000 Da.

Preliminary Experiments to Determine the Optimal Extraction Method

Batch experiments were used to determine the sorption of Ag^+ and AgNPs as a function of pH and Ag concentration. Preliminary experiments to determine the rate of sorption by the soil as a function of time revealed that there was little change in solution concentration after 120 min (Supplemental Fig. S1). Comparing different extractants revealed that 0.1 M KNO_3 resulted in measurable partitioning of Ag between the solid and solution phase (Supplemental Fig. S2). Thereafter, all experiments used 0.1 M KNO_3 agitated with soil for 120 min.

Table 1. Properties of the soils used in the extraction experiments and pot trials. Values in brackets represent the standard error of the mean (*n* = 3).

Property	Templeton loamy sand†	Granular sandy loam‡
Sand/silt/clay (%)	76/20/4	15/60/25
pH (H_2O)	5.1	6.0
CEC ($cmol_c\ kg^{-1}$)§	12.3	22
C (%)	3.3 (0.03)	2.1
N (%)	0.3 (0.00)	0.2
P (mg kg^{-1})	732 (11)	3,414 (26)
S (mg kg^{-1})	383 (6)	491 (6)
Ca (mg kg^{-1})	3,329 (58)	4,147 (117)
Mg (mg kg^{-1})	3,426 (71)	2,400 (95)
K (mg kg^{-1})	2541 (279)	1,951 (59)
Cd (mg kg^{-1})	0.13 (0.00)	1.5 (0.03)
Zn (mg kg^{-1})	70 (2)	173 (10)
Cu (mg kg^{-1})	5 (0)	65 (1)
B (mg kg^{-1})	7.3 (1.0)	33 (0)
Fe (mg kg^{-1})	17,727 (353)	44,606 (96)
Mn (mg kg^{-1})	357 (20)	1,226 (12)
Ag (mg kg^{-1})	<2	<2

† Data from Simmler et al. (2013).

‡ Data from Al Mamun et al. (2016).

§ CEC, cation exchange capacity.

Silver Sorption as a Function of Concentration and pH

Centrifuge tubes (50 mL) were filled with 5 g of soil. Solutions (30 mL of 0.1 M KNO_3) were added containing Ag at concentrations of 0 (control), 9, 19, 38, 75, 150, and 300 mg L^{-1}. Silver was added as either Ag^+ (as $AgNO_3$) or AgNPs. The solution pH was adjusted to values ranging from 2.8 to 8.3 after addition of the sorbent by adding 0, 0.1, 0.2, and 0.3 mL HNO_3 (BDH ARISTAR nitric acid 70%, diluted 1:10) or 0.05, 0.1, 0.2, or 0.4 mL KOH (BDH AnalaR KOH 2 M) to decrease or increase pH, respectively. Tubes were placed on an end-over-end agitator (20 rpm) for 2 h, then centrifuged for 20 min at 4700 rpm. The supernatants were separated and centrifuged again using Eppendorf tubes at 13,400 rpm for 30 min and acidified using 1 mL of concentrated HNO_3.

Extractable Silver in Incubated Soils

Soils were spiked with either 1000 mg kg^{-1} Ag^+ (added as $AgNO_3$) or 1000 mg kg^{-1} Ag (added as AgNPs), and 2.5-g portions were weighed into 45 centrifuge tubes (50 mL). The moisture content of the soils was adjusted to 30% (w/w; i.e., 30 g H_2O, 70 g oven-dried soil). Three tubes were extracted immediately with 15 mL of 0.1 M KNO_3, using the abovementioned methods. The remaining tubes were divided into three lots (21 tubes each) and placed either in a refrigerator at 4°C, in a laboratory cupboard at room temperature (20°C), or in an incubator at 35°C. Three tubes from each treatment were extracted using KNO_3 after 1, 2, 4, 8, and 16 d.

Response of *Lolium perenne* to Silver Ions and Silver Nanoparticles

Portions (5 kg) of the TLS were spiked with either Ag^+ ($AgNO_3$) or AgNP to give Ag concentrations of approximately 19, 39, 78, 156, 312, 625, 1250, 2500, 5000, 10,000 mg kg^{-1}. Control soil without spiking was set aside. The spiking was achieved by preparing the 10,000 mg kg^{-1} using the silver salts and then diluting the mixture with unspiked soil. Subsamples were taken for chemical analyses to determine actual concentrations. Spiked soils were then placed into three 1.5-L pots (n = 3 = number of replicates), moistened to field capacity, and left for 6 wk to equilibrate in the greenhouse facility at Lincoln University. Some 50 seeds of *L. perenne* were sown into each pot allowed to grow for 53 d until a dense sward formed over the controls. Pots were watered once daily to field capacity. The day and night temperatures during the experimental period were 12 and 22°C, respectively. The aerial portions of the *L. perenne* were removed 2 cm above the soil to reduce the risk of contamination with soil particles. Leaf material was thoroughly rinsed in deionized water and then dried at 65°C until a constant weight was obtained. The dry weights were taken and the tubers were ground using a Retch ZM200 grinder and stored in sealed plastic vials.

Uptake of Silver Ions and Silver Nanoparticles by a Selection of Vegetables

Portions (100 kg) of soil were spiked to 70 mg kg^{-1} with either Ag^+ (as $AgNO_3$) or AgNPs. A further 100 kg was set aside as a control. The soils were placed into 135 pots (1.5 L) and placed in the greenhouse at Lincoln University for 6 wk to equilibrate. In December 2016, the pots were planted with either carrot, radish, leek, lettuce, parsley, rocket, beetroot, silverbeet (chard), or spinach. There were five replicates for each treatment, giving a total of 15 pots for each species. Pots were watered daily to field capacity. When mature, the plants were harvested and the edible portions excised and weighed. The harvest dates were February 2017 (carrot), January 2017 (radish), May 2017 (leek), March 2017 (lettuce), April 2017 (parsley and rocket), February 2017 (silverbeet), and January 2017 (spinach). The edible portions were washed thoroughly in deionized water. Root vegetables (carrots, radish, and beetroot) were peeled. The edible portions were dried, weighed again, ground using a Retch ZM200 grinder, and stored in sealed plastic vials.

Chemical Analyses

For both plants and soils, pseudo-total elemental analyses were performed using microwave digestion in 8 mL of Aristar HNO_3 (±69%), diluted with milliQ water to a volume of 25 mL and filtered using Whatman no. 52 filter paper (pore size = 7 μm). Extract solutions were measured directly. The concentrations of Ag together with other elements (Cd, Ca, Mg, K, P, S, B, Cu, Zn, Cr, Ni, and Pb) were determined using inductively coupled plasma optical emission spectrometry (ICP–OES Varian 720 ES) in soils (Kovács et al., 2000) and in plants (Gray et al., 1999). Reference soil and plant material (International Soil analytical Exchange, ISE 921, and International Plant analytical Exchange, IPE 100) from Wageningen University, the Netherlands, were analyzed for quality assurance. Recoverable concentrations were 90 to 106% of the certified values.

For the batch extraction experiments, the solution Ag concentration was determined using a Shimadzu 6400 flame atomic absorption spectrophotometer (FAAS). A parallel ICP–OES analysis was performed on randomly selected samples from the bulk samples to confirm the results of the FAAS.

These analyses only permitted the determination of total Ag, which was the sum of AgNPs and Ag^+ in the solutions, soils, and plants. Information about the fate of AgNPs was obtained by comparing chemical properties (solubility) of AgNPs with Ag^+.

Data Analysis

The distribution of Ag between the solid and solution phase, $K_{D,}$ was calculated from batch experiments following the method of Simmler et al. (2013):

$$K_D = \frac{\text{Ag adsorbed } \left(\text{mg kg}^{-1}\right)}{\text{Ag in solution } \left(\text{mg L}^{-1}\right)} = \frac{\left[C_{\text{Ag }Y/\text{SO}} - \left(C_{\text{Ag }Y/\text{pH }X} - C_{\text{no Ag }Y/\text{pH }X}\right)\right]\left(\text{mg L}^{-1}\right) \times \frac{0.03\text{ L}}{0.005\text{ kg}}}{\left(C_{\text{Ag }Y/\text{pH }X} - C_{\text{no Ag }Y/\text{pH }X}\right)\left(\text{mg L}^{-1}\right)}$$

where $C_{\text{Ag }Y/\text{pH }X}$ is the Ag solution concentration in the treatment in which the sorption is measured. $C_{\text{no Ag }Y/\text{pH }X}$ is the Ag solution concentration in the treatment with the corresponding pH, without Ag spiking, and $C_{\text{Ag }Y/\text{SO}}$ is the Ag solution concentration in the corresponding spiked treatment without addition of sorbent.

Data were tested for normality before analysis. Log-normal data were log-transformed. Significant differences were determined using ANOVA with Fisher's LSD post-hoc test to compare means using Minitab 17. The level of significance was 0.05.

Results and Discussion

Figure 1 shows the sorption of AgNPs and Ag^+ by the TSL and GSL as a function of pH. The results show that at a given pH, the K_D value was tenfold higher for AgNPs than for Ag^+. This indicates that Ag^+ is likely to be more mobile and therefore more soluble and plant available than AgNPs. These findings are in contrast with those of Wang et al. (2018), who reported that the sorption of AgNPs onto 10 soils was generally lower than the corresponding sorption of Ag^+ ions. These differences may be due to coating of the AgNPs, our study used trisodium citrate, whereas Wang et al. (2018) used PVP. Similarly, Rahmatpour et al. (2017) reported that PVP AgNPs had similar sorption properties to Ag^+ ions in four calcareous soils. Differences in the surface chemistry of the AgNPs may have led to differences in specific adsorption processes and highlights the importance of the surface coating on the environmental fate of nanoparticles in general (Klitzke et al., 2015).

The reduced sorption of both AgNPs and Ag^+ ions at higher concentrations is consistent with other studies (Klitzke et al., 2015; Rahmatpour et al., 2017; Wang et al., 2018) and is likely due to saturation of binding sites in soil (Robinson et al., 2009). This mechanism may explain the significantly higher sorption of AgNPs compared with Ag^+ ions in our study. Although the concentrations of AgNPs and Ag^+ ions were similar on a mass ratio concentration (i.e., mg L^{-1} in solution), the *molar* concentration of AgNPs is lower than Ag^+ ions at a similar mass ratio concentration. The nanoparticles used in this study had an average radius of 12.5 nm, some 73 times larger than that of Ag^+ ions (0.172 nm). Therefore, the molar concentration of AgNPs will be some 380,000 times lower than that of Ag^+ ions at the same mass ratio concentration. On a molar basis, the sorption of AgNPs would be lower than for Ag^+ ions.

For both AgNPs and Ag^+, K_D increases with increasing pH. The behavior of Ag^+ ions is similar to that of other cations in soil solution, where sorption increases at high solution pH because increasing negative charge on soil colloids minimizes repulsion between like-charged Ag^+ ions and the surface. The manner in which Ag^+ ions sorb to the surface is secondary because an increased negative charge on soil colloids increases outer-sphere and inner-sphere adsorption (Robinson et al., 2009). The reason for the increase in AgNP sorption at higher pH values is less clear. Given that the AgNPs in this experiment were coated with citrate, which acquires a negative charge, we hypothesized that sorption may have been reduced at higher pH values. This increase in sorption may be explained if the citrate coating was removed (e.g., during the extraction process), or if the citrate coating itself became positively charged due to the sorption of cations. Although we did not aim to elucidate the sorption mechanisms in this study, our findings are consistent with those of Wang et al. (2018), who reported that PVP AgNPs primarily bound to iron oxides; in our results, the GSL, which is rich in iron oxides (Table 1), sorbed some fivefold more AgNPs than the TLS at similar pH values.

Figure 2 shows the effect of incubation on the solubility of AgNPs and Ag^+ ions at 4, 20, and 35°C. For Ag^+ ions, sorption over 16 d significantly increased, with the largest increases occurring at the highest temperature. This is consistent with many studies that have demonstrated that trace elements become less soluble over time as a function of reaction period (Mclaughlin et al., 1996) and occur due to increasing specific adsorption, occlusion by iron and aluminum oxides, as well as sorption into soil colloids (Robinson et al., 2009). In contrast, incubation of AgNPs decreased their sorption onto the test soil, with the largest decreases in sorption occurring at the highest incubation temperature (35°°C). Given the solubility of Ag^+ is greater than AgNPs (Fig. 1), the increase in solubility over time is consistent with the degradation of AgNPs into Ag^+ ions, a result also reported by McShan et al. (2014). The significant decrease in sorption even with the AgNPs incubated at 4°C indicates that over the course of several months all the AgNPs will probably transform into Ag^+ ions. Therefore, the solubility of Ag in soils contaminated with AgNPs may increase with time. These findings indicate that the results of other studies may change dramatically if they are conducted on aged, rather than freshly spiked soil.

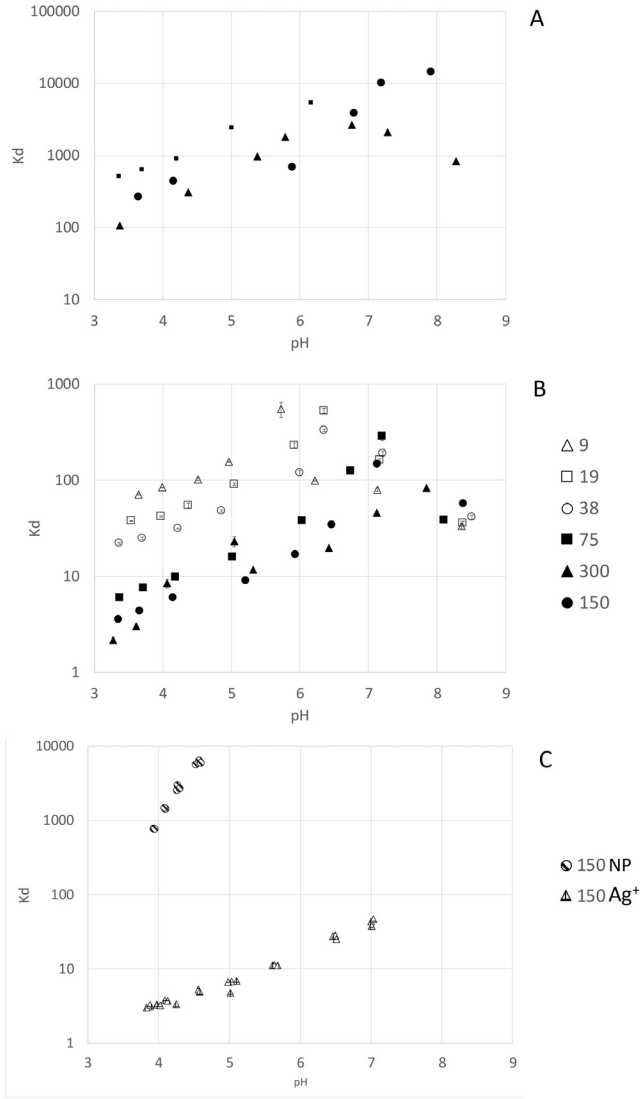

Fig. 1. Distribution coefficients (K_D = sorbed/solution concentration quotients) as a function of pH for (A)Ag nanoparticles (NPs) in the Templeton silt loam, (B) ionic silver (Ag^+) in the Templeton silt loam, and (C) both NPs and Ag^+ in the Pukekohe granular soil. The legend indicates the initial concentrations in the ambient solution (mg L^{-1}). For Graphs A and B, bars indicate the standard error of the mean (n = 3).

Figure 3A shows the effect of AgNPs and Ag^+ on the biomass index (treatment/control biomass quotient) of *L. perenne*. At soil concentrations <100 mg Ag kg^{-1}, the Ag^+ ion treatment had a significantly higher biomass index compared to the AgNPs. Given that at Ag^+ ion concentrations of <10 mg kg^{-1},

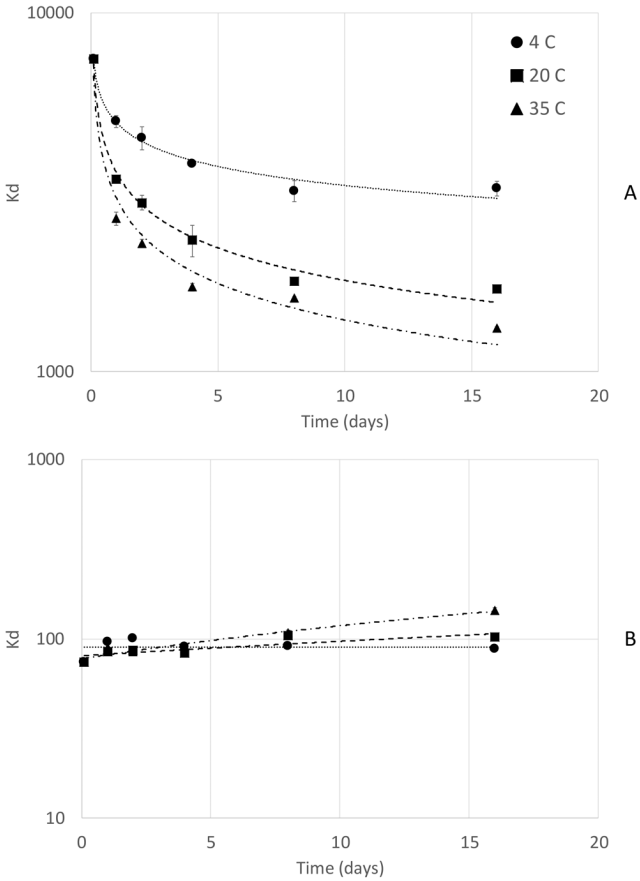

Fig. 2. Change in the distribution coefficient (K_D = sorbed/solution concentration quotients) as a function of incubation time at 4, 20, and 35°C for (A) nanoparticles and (B) ionic silver in the Templeton silt loam. Error bars represent the standard error of the mean (n = 3).

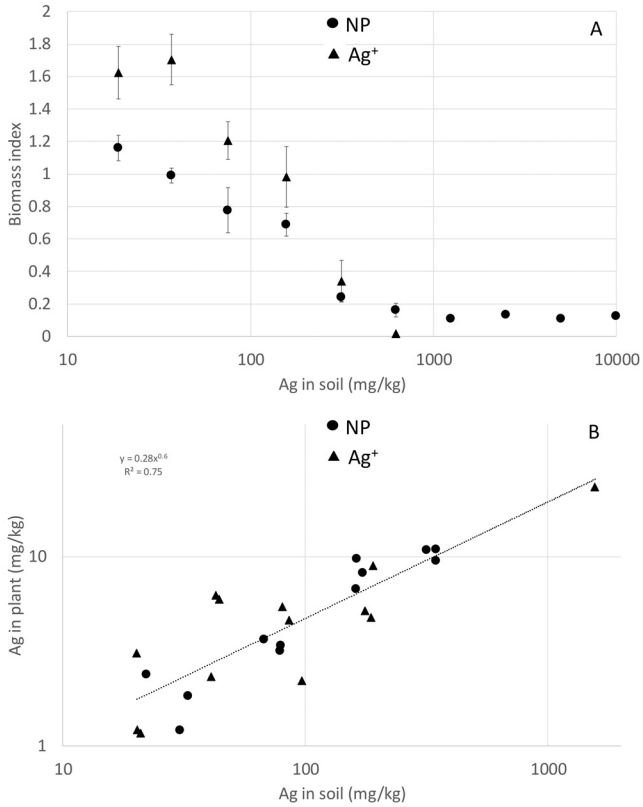

Fig. 3. (A) Plant biomass index (treatment/control mass quotient) as a function of Ag concentration in soil (Templeton loamy silt). Bars represent the standard error of the mean (n = 3). (B) Leaf Ag concentration as a function of Ag concentration in soil. The line shows the best fit for all points. There were no significant differences in plant uptake between Ag nanoparticles (NPs) and Ag^+.

the treatments were higher than the controls (i.e., the biomass index > 1), these lower treatments had a stimulatory effect on plant growth. This may be due to the nitrate that was added as a counter-ion to the Ag^+ ions, rather than the Ag^+ itself. At concentrations >625 mg kg^{-1} there was no growth in the Ag^+ ion treatments, whereas there were small but measurable growth of the *L. perenne* in the AgNPs treatments. Figure 3B shows that there were no differences between the AgNPs treatment and the Ag^+ treatment with regard to Ag uptake by *L. perenne*. If there were a significant transformation of AgNPs into Ag^+ ions (Fig. 2), there would be few material differences in the treatments during the experimental period as the soils were incubated for 6 wk at 20°C prior to planting. The results show that at Ag concentrations (AgNPs and Ag^+ ions) of <100 mg kg^{-1} (where the biomass was not significantly different from the control), there was measurable plant uptake, with a bioaccumulation coefficient (plant/soil concentration quotient) of 0.01 to 0.1, with the maximum plant concentration ~5 mg kg^{-1} occurring in soils with ~100 mg Ag kg^{-1}.

Figure 4 shows the Ag uptake of nine vegetables grown in the TLS without added Ag or spiked with AgNPs or Ag^+ ions (at 70 mg Ag kg^{-1}) and incubated for 48 h before planting. Except for lettuce, all the treatments accumulated significantly more Ag than the control. On average, the control plants took up <0.5 mg Ag kg^{-1}, and plants grown in soils spiked with AgNPs or Ag^+ ions taking up an average of 3.8 and 3.5 mg kg^{-1}, respectively, with no significant difference between the AgNP

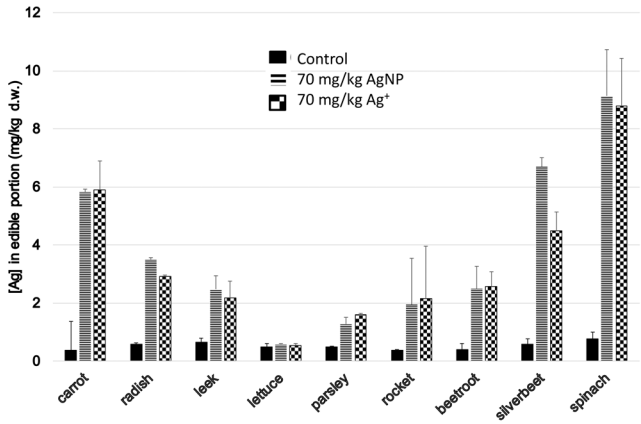

Fig. 4. Silver concentration in the edible portions of plants grown in the Templeton silt loam either without silver or spiked with 70 mg kg^{-1} either Ag nanoparticles (AgNPs) or ionic Ag (Ag^+). Bars represent the standard error of the mean (n = 5).

and Ag^+ concentrations across all species. However, there were significant differences in Ag uptake between species, with the highest concentrations (5–9 mg kg^{-1}) occurring in carrots, silverbeet, and spinach. There were no significant differences in plant Ag uptake between the AgNPs and Ag^+ ions treatments, except for radish and silverbeet, where there were small but significant differences, with more Ag taken up in the plants growing in soils spiked with AgNPs.

Table 2 shows the mass of each vegetable that a 70 kg individual would need to consume to exceed the tolerable daily

Table 2. Mass required to exceed the tolerable daily intake of 0.005 mg kg d^{-1} for a 70-kg person.

Crop	Control	Ag nanoparticles	Ag^+
	g dry matter		
Carrot	921	60	59
Radish	603	100	120
Leek	538	141	161
Lettuce	714	603	648
Parsley	700	273	219
Rocket	921	179	162
Beetroot	875	141	136
Silverbeet	603	52	78
Spinach	449	38	40

intake (TDI) for Ag, which is set at 0.005 mg kg^{-1} d^{-1} (USEPA, 2019). For all vegetables except carrots, silverbeet, and spinach in the Ag treatments, an individual is unlikely to exceed the TDI following a normal diet. For spinach, the TDI may be exceeded by consuming as little as 38 g (dry matter). These results indicate that there is a potential human health risk through consuming vegetables grown in Ag-contaminated soil. The vegetables in these experiments were peeled and washed. It is likely that more Ag would be consumed on unpeeled or poorly washed vegetables as significant amounts of Ag may be consumed via dust particles that are attached to the plant (Al Mamun et al., 2017).

Conclusions

The soil sorption of AgNPs was significantly greater than Ag^+, and both moieties were preferentially retained on the soils' surfaces at lower concentrations and at higher pH values. Similarly, at equal mass concentrations, Ag^+ was some tenfold more soluble than AgNPs, whereas the reverse was true if molar concentrations were compared. Although we do not have conclusive proof, there was strong evidence that AgNPs were transformed into Ag^+ during the course of these experiments. Therefore, regarding citrate-coated AgNPs, the environmental impact of their release may be largely determined by the equivalent mass concentration of Ag^+, into which they will ultimately transform. There was a large variation between plant species in Ag uptake, with some members of the Amaranthaceae (spinach and silverbeet) accumulating sufficient Ag to present a risk to human health. Given the widespread interest in AgNPs, future work could focus on the use of cyclic voltammetry as a probing tool to investigate dissolution of AgNPs and Ag L-edge X-ray absorption near edge structure (XANES) spectroscopy to elucidate to what extent AgNPs are dissolved into Ag^+ and retained on the mineral surfaces. The role of citrate in the binding mechanism of AgNPs could be explored in the future using attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) as citrate is infrared active.

Supplemental Material

Supplemental Fig. S1 and S2 show the solubility of Ag and AgNPs as a function of extraction time and chemistry. Supplemental Table S1 describe the preparation of the AgNPs. Supplemetnal Table S2 gives the fresh weight and dry weight of the *Lolium perenne* in soils spiked with either AgNPs or Ag^+.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the Centre for Integrated Biowaste Research (CIBR) for funding this project. The authors acknowledge careful revision and editing by the journal's associate editor, Markus Graefe.

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